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Foliar Flavonoid Composition in Japanese *Cirsium* Species (Compositae), and Their Chemotaxonomic Significance

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Foliar flavonoids of 17 *Cirsium* taxa were isolated and identified, and the flavonoid profiles of five of them were newly determined. Among ten flavone and flavonol glycosides characterized from the above taxa, pectolinarin frequently accompanied by linarin showed the most wide distribution occurring in 12 taxa. On the other hand, luteolin 7-*O*-glucoside was detected from *C. chikushiense*, *C. suffultum* and *C. yakushimense*, and apigenin and luteolin 5-*O*-glucosides from *C. magofukui* and *C. sieboldii*. 3-*O*-Galactoside and 3-*O*-arabinosylgalactosides of kaempferol and quercetin, and vicenin-2 were isolated from *C. babanum* and *C. gratiosum* as minor components, respectively. The finding of vicetin-2 in the genus *Cirsium* was done for the first time.

Major flavonoids found from 32 *Cirsium* species were divided into three types by the chemical structures, i.e., **A**: polymethylated flavones possessing 6-methoxyl group except linarin, **B**: flavone 5-*O*-glycosides and **C**: flavone 7-*O*-glycosides. **A**-type was additionally subdivided by the position and number of methoxyl group in aglycones into **A-1**: pectolinarigenin, **A-2**: cirsimaritin, and **A-3**: cirsilineol and cirsiliol.

Intrapopulational flavonoid variation was not observed, but interpopulational variation occurred in some species. Any phyletic relationship is not necessarily present in the taxa of each flavonoid type, so that their chemical characters may be useful for the elucidation of intraspecific variation of each species rather than interspecific, intersectional or intersubsectional comparisons in the genus *Cirsium*.

Introduction

The genus *Cirsium* (Compositae) consists of about 300 species in the Northern Hemisphere. In Japan, 64 species are widely distributed from the sea shore to high mountains (Kadota 1995). Flavonoid compounds in the leaves of Japanese *Cirsium* species have been isolated and identified by Morita and co-workers (Morita 1976; Morita and Shimizu 1963; Morita et al. 1964, 1965, 1973; Nakaoki and Morita 1959, 1960). Some methylated flavone *O*-glycosides, e.g., pectolinarin (5,7-dihydroxy-6,4'-dimethoxyflavone 7-*O*-rutinoside), linarin (5,7-dihydroxy-4'-methoxyflavone 7-*O*-rutinoside), cirsimarin (5,4'-dihydroxy-6,7-dimethoxyflavone 4'-*O*-glucoside), cirsilineol (5,4'-dihydroxy-6,7,3'-trimethoxyflavone) 4'-*O*-glucoside and cirsiliol (5,3',4'-trihydroxy-6,7-dimethoxy-

flavone) 4'-*O*-glucoside, have been found in addition to commonly occurring luteolin 7-*O*-glucoside, apigenin 7-*O*-rutinoside and so on. On the other hand, another flavonoid type containing flavone 5-*O*-glycosides, apigenin and luteolin 5-*O*-glucosides was found by Iwashina et al. (1989). The occurrence of their glycosides are unusual since the 5-hydroxyl group would resist against glycosylation by the hydrogen bonding with the adjacent 4-carbonyl group (Harborne 1967, Iwashina et al. 1995).

In this paper, we describe the flavonoid composition of 17 *Cirsium* taxa. Of their taxa, five species, *C. babanum* Kitam., *C. chikushiense* Koidz., *C. gratiosum* Kitam., *C. senjoense* Kitam. and *C. spicatum* (Maxim.) Matsum., were analyzed for flavonoids for the first time.

Materials and Methods

Plant materials *Cirsium* species which were analyzed in this experiment were collected from the localities cited below.

C. babanum Koidz.: between Shirouma-ooike – Renge Hotspring, near Mt. Shirouma, Niigata Pref. (25 individuals), collected by Y. Kadota.

C. bitchuense Nakai: Mt. Ibuki, Gifu Pref. (3 individuals), collected by T. Ueno.

C. chikushiense Koidz.: Uchinoura, Kagoshima Pref. (1 individual), collected by T. Iwashina; Mt. Noma, Kagoshima Pref. (11 individuals), collected by Y. Kadota.

C. gratiosum Kitam.: Mt. Senmai and Mt. Akaishi, Shizuoka Pref. (each 1 individual), collected by Y. Kadota and T. Iwashina.

C. japonicum Fisch. ex DC.: Shinojima Isl. and Obara, Aichi Pref. (each 3 individuals), collected by T. Ueno; Ooshirakawa, Niigata Pref. (3 individuals), Sekizaki, Ooita Pref. (1 individual) and Tazawa, Akita Pref. (1 individual), collected by T. Iwashina; and Tobishima Isl., Yamagata Pref. (1 individual), collected by S. Domon.

C. kagamontanum Nakai: Mizutaki, Fukui Pref. (7 individuals), collected by T. Wakasugi; and Mt. Hira (Bunagatake), Shiga Pref. (1 individual), collected by T. Ueno.

C. magofukui Kitam.: Shouke-Valley, Gifu Pref. (9 individuals), collected by T. Ueno; Imajo, Fukui Pref. (18 individuals), collected by Y. Kadota; and Mt. Ibuki, Shiga Pref. (10 individuals), collected by T. Ueno and T. Iwashina.

C. microspicatum Nakai var. *kiotense* Kitam.: Aburasaka Pass, Fukui Pref. (6 individuals) and Suizawa (Mizusawa) Pass, Shiga Pref. (1 individual), collected by T. Ueno; and Katsuyama, Fukui Pref. (7 individuals), collected by Y. Kadota.

C. microspicatum Nakai var. *microspicatum*: Hidamiyada, Gifu Pref. (1 individual), collected by T. Ueno; Kamikouchi, Nagano Pref. (7 individuals), collected by T. Iwashina.

C. nipponicum (Maxim.) Makino var. *incomptum* (Maxim.) Kitam. ex Ohwi: Hatanagi, Shizuoka Pref. (1 individual), collected by Y. Kadota and T. Iwashina.

C. otayae Kitam.: between Shirouma-ooike – Renge Hotspring, near Mt. Shirouma, Niigata Pref. and Mt. Kashima-yarigatake, Nagano Pref. (each 20 and 21 individuals), collected by Y. Kadota.

C. senjoense Kitam.: Mt. Akaishi, Shizuoka Pref. (2 individuals), collected by Y. Kadota and T. Iwashina.

C. sieboldii Miq.: Tsukude, Aichi Pref., Mt. Mikuni, Makino-cho, Shiga Pref. and Yohka-ichi, Shiga Pref. (each 7, 3 and 2 individuals), collected by T. Ueno; and Mt. Hiruzen and Saijo, Okayama Pref. (each 10 individuals), collected by Y. Kadota.

C. spicatum (Maxim.) Matsum.: Takanomori, Kumamoto Pref. (1 individual), collected by T. Iwashina.

C. suffultum (Maxim.) Matsum.: Mt. Osuzu, Miyazaki Pref. (2 individuals), Uchinoura and Mt. Kaimon, Kagoshima Pref. (each 1 and 2 individuals), collected by T. Iwashina.

C. yakushimense Masam.: Yaku Isl., Kagoshima

Pref. (4 individuals), collected by Y. Kadota.

C. yezoense (Maxim.) Makino: Nekura Valley, Fukui Pref. and Sasamata, Gifu Pref. (each 3 and 2 individuals), collected by T. Ueno; and Mt. Houonji, Fukui Pref. (1 individual), collected by T. Wakasugi.

Voucher specimens are deposited in the Herbarium, National Science Museum, Tokyo (TNS).

Isolation of flavonoids Fresh leaves were extracted with methanol, filtered and evaporated to dryness in vacuo. The residue was applied to preparative paper chromatography (PPC) using solvent systems, BAW, 15% AcOH and then BEW (see Table 1). Finally, the flavonoids were purified by a Sephadex LH-20 column (I.D. 10 × 200 mm) using 70% MeOH. Most major flavonoid glycosides were obtained as pale yellow needles. In case of pectolinarin, it was easily crystallized in crude methanol extract, after standing overnight in the cold.

Identification of flavonoids The flavonoids were identified by UV spectral analysis according to the methods by Mabry et al. (1970), fast atom bombardment mass spectra (FAB-MS) using nitrobenzyl alcohol (NBA), ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra using dimethylsulfoxide-d_6 (DMSO-d_6), identification of their hydrolysates by the acid hydrolysis according to the methods by Hayashi et al. (1989) and by PC and HPLC comparisons with authentic specimens.

High performance liquid chromatography (HPLC) HPLC separation of flavonoid glycosides were performed with TSKgel ODS-TM (I.D. 4.6 × 150 mm) column, at flow-rate; 1.0 ml/min, detection; 345 nm

Table 1. Paper chromatographic data of flavonoid glycosides from the leaves of five Japanese *Cirsium* species which were newly analyzed

Flavonoids	Rf values				Colors	
	BAW	BEW	15%AcOH	5%AcOH	UV	UV/NH_3
Pectolinarigenin 7-rutinoside	0.48	0.53	0.55	0.28	dark purple	dark purple
Acacetin 7-rutinoside	0.43	0.46	0.41	0.18	dark purple	dark purple
Quercetin 3-galactoside	0.48	0.52	0.40	0.16	dark purple	yellow
Quercetin 3-arabinogalactoside	0.22	0.18	0.54	0.33	dark purple	yellow
Kaempferol 3-galactoside	0.59	0.66	0.47	0.19	dark purple	dark greenish yellow
Kaempferol 3-arabinogalactoside	0.28	0.32	0.61	0.42	dark purple	dark greenish yellow
Luteolin 7-glucoside	0.34	0.38	0.10	0.03	dark purple	yellow
Vicenin-2	0.19	0.14	0.56	0.30	dark purple	dark yellow

BAW = n-BuOH/AcOH/water (4:1:5, upper phase), BEW = n-BuOH/EtOH/water (4:1:2.2), 15%AcOH = AcOH/water (15:85) and 5%AcOH = AcOH/water (5:95).

and eluent; $CH_3CN/H_2O/H_3PO_4$ (22:78:0.2) according to Hayashi et al. (1989).

Results

Identification of flavonoids from Cirsium *species*

Ten flavone and flavonol glycosides were isolated and sufficiently characterized. Of their glycosides, identification of eight, which were isolated from the newly surveyed five *Cirsium* species (*C. babanum, C. chikushiense, C. senjoense, C. spicatum* and *C. gratiosum*) are described.

Pectolinarigenin 7-*O*-rutinoside (pectolinarin) UV spectra of pale yellow needles which could be easily obtained from crude methanol extract of *Cirsium babanum, C. gratiosum, C. senjoense, C. spicatum* and so on, showed the presence of free 5-hydroxyl and substituted 7- and 4'-hydroxyl groups (Table 2; Mabry et al. 1970). Moreover, Band II (276 nm) in methanol suggested the substitution of 6- and/or 8-position. On acid hydrolysis, the original glycoside liberated glucose, rhamnose and an aglycone, of which UV spectra indicated the presence of free 5- and 7-hydroxyl groups showing the attachment of sugars to 7-position. The FAB-MS exhibited $[M-H]^-$ at *m/z* 621, calculated for $C_{29}H_{34}O_{15}$ showing the attachment of each 1 mol glucose and rhamnose to 5,7-dihydroxy-6 (or 8), 4'-methoxyflavone. Moreover, the presence of a methoxyl group on 6-position, but not on 8-position, was shown by the absence of signal corresponding to 6-proton on ^{1}H-NMR. Finally, the original glycoside was identified as pectolinarigenin 7-*O*-β-D-rutinoside (pectolinarin, see Fig. 1) by direct PC and HPLC comparison with authentic specimen (Table 1).

^{1}H-NMR data was as follows.

^{1}H-NMR (270MHz, DMSO-d_6) δ 8.05 (2H, *d*, *J* = 8.9Hz, H-2',6'), 7.17 (2H, *d*, *J* = 8.9Hz, H-3',5'), 6.94 (2H, *s*, H-8 and H-3), 5.47 (1H, *d*, *J* = 5.3Hz, glucosyl anomer), 5.25 (1H, *s*, rhamnosyl anomer), 5.2–3.1 (10H, *m*, sugar protons), 3.87 and 3.77 (each 3H, *s*, OMe × 2), 1.06 (3H, *d*, *J* = 6.3Hz, rhamnosyl Me).

Pectolinarin has been isolated from many Japanese *Cirsium* species, e.g., *C. brevicaule* A. Gray, *C. japonicum, C. pectinellum* A. Gray, *C. yezoense* and so on, as a major compound (Iwashina et al. 1988, Morita et al. 1964, Nakaoki and Morita 1959, 1960, see Table 4).

Acacetin 7-*O*-rutinoside (linarin). UV spectra of the flavonoid glycoside, which was found with pectolinarin from some *Cirsium* species (Table 3), showed the presence of free 5-hydroxyl and substituted 7- and 4'-hydroxyl groups (Table 2, Mabry et al. 1970). Acacetin (5,7-dihydroxy-4'-methoxyflavone), glucose and rhamnose were liberated by acid hydrolysis. Finally, the original glycoside was identified as acacetin 7-*O*-rutinoside (linarin) by direct PC and HPLC comparison with authentic specimen (Table 1).

Linarin has also been found from many *Cirsium* species and accompanied with pectolinarin in most taxa except *C. purpuratum* (Maxim.) Matsum. and *C. spinosum* Kitam. (Morita et al. 1964, Nakaoki and Morita 1959).

Quercetin 3-*O*-galactoside (hyperin) and kaempferol 3-*O*-galactoside (trifolin). The flavonoids were isolated from *C. babanum* as minor components. Quercetin and galactose, and kaempferol and galactose were found to be acid hydrolysates, respectively. UV spectra of the original glycosides showed the attachment of galactose to 3-position of quercetin and kaempferol (Table 2; Mabry et al. 1970). It was shown by PC and HPLC comparison with authentic specimens that the original glycosides were quercetin 3-*O*-galactoside (hyperin) and kaempferol 3-*O*-galactoside (trifolin).

Though flavonol glycoside (kaempferol 3-*O*-glucoside) from four *Cirsium* species, *C. lucens* Kitam. var. *opacum* Kitam., *C. microspicatum* var. *kiotense, C. nipponicum* var. *incomptum* and *C. yezoense* as a minor component has been reported (Iwashina et al. 1988), hyperin and trifolin were found from *Cirsium*

Table 2. UV spectral data of flavonoid glycosides from the leaves of five Japanese *Cirsium* species which were newly analyzed

Flavonoids	λ max (nm)					
	in MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
Pectolinarigenin	276	296	285	286	275	275
7-rutinoside	328	376*	300	299	329	330
			356	349		
			386sh	386sh		
Acacetin	268	287	275	277	269	269
7-rutinoside	325	367*	300	299	324	330
			344	338		
			380	381		
Quercetin	257	273	274	268	273	261
3-galactoside	358	328	427	299	324	373
		410**		362	391	
				397		
Quercetin	257	274	275	269	274	262
3-arabinogalactoside	359	332	435	301	321	378
		408**		364	391	
				400		
Kaempferol	267	275	274	275	274	267
3-galactoside	348	327	304	302	307	352
		401**	354	351	384	
			393	390		
Kaempferol	267	275	274	275	274	267
3-arabinogalactoside	353	326	305	303	312	355
		403**	357	350	385	
			399	397		
Luteolin	254	266	273	274	258	259
7-glucoside	348	388**	427	293	399	372
				357		
				386		
Vicenin-2	273	282	280	280	282	276
	332	334	306	304	392	323
		401**	353	346		
			383sh	383sh		

*Remarkable decrease in intensity relative to MeOH spectrum, and **remarkable increase in intensity. sh = shoulder.

species for the first time.

Quercetin 3-O-arabinosylgalactoside and kaempferol 3-O-arabinosylgalactoside. Their glycosides were also found from *C. babanum* as minor compounds. UV spectra of their glycosides showed the presence of free 5,7,3',4'-tetraOH (former) and 5,7,4'-triOH (latter) and a substituted 3-OH (Table 2; Mabry et al. 1970). Quercetin or kaempferol, arabinose and galactose were liberated by acid hydrolysis. Their glycosides were accord-

ingly considered to be quercetin and kaempferol 3-*O*-arabinosylgalactosides, respectively. The quercetin and kaempferol glycosides from other families, *Hydrocotyle vulgaris* L. (Umbelliferae) (Hiller et al. 1979) and *Lysichiton camtschatcense* (L.) Schott (Araceae) (Williams et al. 1981) have been reported, respectively.

Luteolin 7-*O*-glucoside. The flavonoid was isolated from *C. chikushiense, C. suffultum* and *C. yakushimense* as a major component. UV spectra of the glycoside showed the presence of free 5-, 3'- and 4'-hydroxyl and a substituted 7-hydroxyl group (Table 2; Mabry et al. 1970). The aglycone and glycosidic sugar, which were liberated by acid hydrolysis, were identified as luteolin and glucose. Finally, the original glycoside was identified as to be luteolin 7-*O*-glucoside by direct PC and HPLC comparison with authentic specimen (Table 1).

Luteolin 7-*O*-glucoside from some Japanese *Cirsium* species, e.g., *C. buergeri* Miq., and also *C. lucens, C. suffultum* and *C. yakushimense* as a major component has been reported (Iwashina et al. 1988, Morita et al. 1964, 1965, 1973, Nakaoki and Morita 1960, see Table 4). In *C. chikushiense*, apigenin and chrysoeriol 7-*O*-glycosides, which were partially characterized, were contained as minor components.

Apigenin 6,8-di-*C*-glucoside (vicenin-2). The flavonoid was found from *C. gratiosum* as a minor component. The glycoside resisted to hot acid hydrolysis, showing that the compound was 6,8-di-*C*-glycosylflavone which attached the same kind of sugar to both 6- and 8-positions (Markham 1982). In UV spectral analysis, it was shown that the presence of free 5-, 7- and 4'-hydroxyl groups (Table 2; Mabry et al. 1970). PC and HPLC data of the original glycoside completely agreed with those of authentic vicenin-2 (Table 1).

In *C. gratiosum*, another minor *C*-glycosylapigenin was present, but could not sufficiently be characterized. *C*-glycosylflavones were found from *Cirsium* species for the first time.

Distribution of flavonoids in Cirsium Among 17 *Cirsium* taxa surveyed in this experiment, twelve involving *C. babanum, C. senjoense, C. spicatum* and *C. gratiosum*, which were newly surveyed for the flavonoid composition, contained pectolinarin as a major flavonoid (Table 3). They accompanied with linarin except two species, *C. bitchuense* and *C. otayae*. Moreover, kaempferol 3-*O*-galactoside and 3-*O*-arabinosylgalactoside, and quercetin 3-*O*-galactoside and 3-*O*-arabinosylgalactoside, were found from *C. babanum*, and vicenin-2 from *C. gratiosum* as minor components, respectively. Rare apigenin and luteolin 5-*O*-glucosides were detected from *C. sieboldii* and *C. magofukui*. Common luteolin 7-*O*-glucoside was found from *C. suffultum, C. yakushimense*, and also from newly surveyed *C. chikushiense* with minor apigenin and chrysoeriol 7-*O*-glycosides.

Discussion

The major flavonoids, which have been isolated from 32 Japanese *Cirsium* taxa (Iwashina et al. 1988, 1989, Morita et al. 1964, 1965, 1973, Morita and Shimizu 1963, Nakaoki and Morita 1959, 1960) were divided into three types, i.e., **A**: polymethylated flavones, which were also 6-substituted except linarin, **B**: rare flavone 5-*O*-glycosides such as apigenin and luteolin 5-*O*-glucosides, and **C**: common flavone 7-*O*-glycosides such as luteolin 7-*O*-glucoside and 7-*O*-glucuronide, and apigenin 7-*O*-rutinoside (Table 4). Moreover, **A**-type was subdivided into three types by the position and number of methylation on aglycones, i.e., **A-1**: pectolinarigenin (5,7-dihydroxy-6,4'-dimethoxyflavone), which accompanied with acacetin (5,7-dihydroxy-4'-methoxyflavone) in most of the taxa, **A-2**: cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone), and **A-3**: cirsilineol (5,4'-dihydroxy-6,7,3'-trimethoxyflavone) which accompanied with cirsiliol (5,3',4'-trihydroxy-6,7-dimethoxyflavone) (Fig. 1). Type **A-1** was most com-

Table 3. Flavonoid composition of Japanese *Cirsium* taxa which were surveyed in this experiment

Taxa	Major flavonoid					Minor flavonoid
	Pe	Ln	A5g	L5g	L7g	
C. sieboldii			+	+		
C. magofukui			+	+		
*C. babanum**	+	+				kaempferol 3-galactoside kaempferol 3-arabinogalactoside quercetin 3-galactoside quercetin 3-arabinogalactoside
C. yezoense	+	+				
C. suffultum					+	
*C. chikushiense**					+	apigenin 7-glycoside chrysoeriol 7-glycoside
C. yakushimense					+	
C. japonicum	+	+				
*C. senjoense**	+	+				
C. otayae	+					
C. nipponicum var. *incomptum*	+	+				
C. microspicatum var. *microspicatum*	+	+				
C. microspicatum var. *kiotense*	+	+				
*C. spicatum**	+	+				
C. bitchuense	+					
*C. gratiosum**	+	+				vicenin-2 *C*-glycosylapigenin
C. kagamontanum	+	+				

*Newly surveyed in this experiment.
Pe = pectolinarigenin 7-*O*-rutinoside (pectolinarin), Ln = acacetin 7-*O*-rutinoside (linarin), A5g = apigenin 5-*O*-glucoside, L5g = luteolin 5-*O*-glucoside and L7g = luteolin 7-*O*-glucoside.

mon and involved 19 *Cirsium* taxa (Table 4), which were classified into various series, subsections or sections according to Kadota (1995). Moreover, pectolinarin from American *Cirsium* species, *C. coloradense* (Rydb.) Cockerell. (Gardner 1973) and *C. foliosum* (Hook.) DC. (Gardner 1974), and European *C. oleraceum* Scop. (Wagner et al. 1960) has also been reported. Types **A-2** and **A-3** from *C.*

Table 4. Flavonoid composition of Japanese *Cirsium*

Flavonoid type				
A			B	C
1	2	3		
C. purpuratum[2)]	*C. maritimum*[6)]	*C. lineare*[3)]	*C. sieboldii*[*,1),8)]	*C. sieboldii*[*,9)]
C. babanum[1)]	*C. kamtschaticum*[*,4)]		*C. magofukui*[1),8)]	*C. lucens*[9)]
C. yezoense[1),3),4)]			*C. kagamontanum*[*,8)]	*C. suffultum*[1),3)]
C. pectinellum[3),4)]			*C. longe-pedunclatum*[8)]	*C. nipponicum* var. *yoshinoi*[5)]
C. brevicaule[5)]				*C. chikushiense*[1)]
C. spinosum[5)]				*C. yakushimense*[1),5)]
C. japonicum[1),2),4)]				*C. inundatum*[*,4)]
C. dipsacolepis[5)]				*C. matsumurae*[5)]
C. inundatum[*,7)]				*C. bitchuense*[*,9)]
C. kamtschaticum[*,3)]				*C. buergeri*[5)]
C. senjoense[1)]				*C. gyojanum*[9)]
C. otayae[1),2)]				
C. nipponicum var. *incomptum*[1),4)]				
C. microspicatum var. *microspicatum*[1),2),4)]				
C. microspicatum var. *kiotense*[1),4),5)]				
C. spicatum[1)]				
C. bitchuense[*,1)]				
C. gratiosum[1)]				
C. kagamontanum[*,1),7)]				

*Occurrence of intraspecific or geographic variations of flavonoid composition. [1)]Present paper, [2)]Nakaoki and Morita (1959), [3)]Morita et al. (1973), [4)]Iwashina et al. (1988), [5)]Morita et al. (1964), [6)]Morita and Shimizu (1963), [7)]Nakaoki and Morita (1960), [8)]Iwashina et al. (1989) and [9)]Morita et al. (1965).

maritimum Makino and *C. kamtschaticum* Ledeb. ex DC. (Iwashina et al. 1988, Morita and Shimizu 1963), and *C. lineare* (Thunb.) Sch.-Bip. (Morita et al. 1973), have been reported.

B- and **C**-types consist of four and 11 taxa, respectively, as mentioned in Table 4. The flavone glycosides in **B-** and **C**-types could be distinguished by the position (5- or 7-) of glycosylation. Since 5-hydroxyl group forms hydrogen bonding with the adjacent 4-carbonyl group, 5-*O*-glycosides rarely occurred in nature (Harborne 1967, Iwashina et al. 1995). Hence it was regarded that 5-*O*-glycosides and 7-*O*-glycosides were phylogenetically different.

Though the intrapopulational flavonoid variation was not observed except in the cases of presumable hybrid derivatives, e.g., *C. magofukui* × *C. microspicatum* var. *kiotense* (Iwashina and Kadota unpublished data), interpopulational flavonoid variation occurred in some species. For example, Morita et al. (1965) isolated luteolin 7-*O*-glucuronide (**C**-type) from *C. sieboldii* which was collected in Yagami, Okayama Pref. On the other hand, we found apigenin and luteolin 5-*O*-glucosides (**B**-type) in other populations, Tsukude, Aichi Pref., Mt. Mikuni, Shiga Pref., Yohka-ichi, Shiga Pref., and Mt. Hiruzen and Saijo, Okayama Pref. as reported in this experiment. In *C. bitchuense*, apigenin 7-*O*-rutinoside (**C**-type) has been reported from Okayama population (Morita et al. 1965), but pectolinarin (**A-1** type) was found from the easternmost population, Mt. Ibuki, Gifu

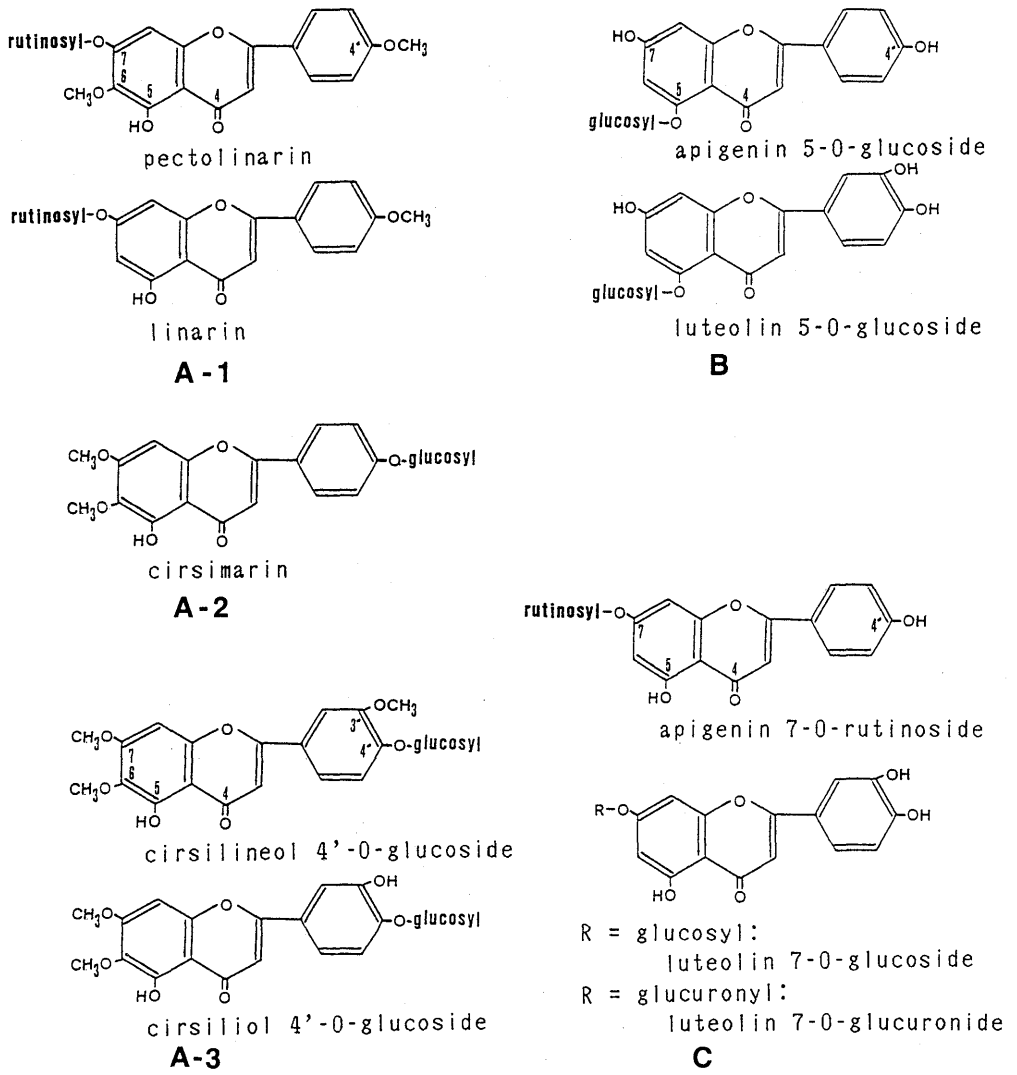

Fig. 1. Major flavone glycosides isolated from Japanese *Cirsium* species.

Pref. (Ueno 1995) by us. As for *C. kagamontanum*, *C. inundatum* Makino and *C. kamtschticum*, **A-1** and **B**, **A-1** and **C**, and **A-1** and **A-2** types were also found from the different populations, respectively (see Materials and Methods, Iwashina et al. 1988, 1989, Morita et al. 1973, Nakaoki and Morita 1960). Moreover, the phyletic relationship is not necessarily present in the taxa of flavonoid types as mentioned in Table 4. However, four species, *C. brevicaule* A. Gray, *C. spinosum*, *C. maritimum* (Morita et al. 1964, Morita and Shimizu 1963) and *C. boninense* Koidz. (Iwashina unpublished data) of subsect. Arenicola native to the coastal region of the Pacific Ocean side of Japan have polymethoxyflavones. On the other hand, another Arenicola species, *C. yakushimense* endemic to Yakushima Isl., Kagoshima Pref., contained common luteolin 7-*O*-glucoside (**C**-type). The species seems to be chemically related to *C. suffultum* and *C. chikushiense*, which are native to Kyushu, rather than other Arenicola species.

Though the flavonoid composition of *Cirsium* species is not sufficiently investigated, the three chemotypes with possible phylogenetic differences are recognized in the genus *Cirsium*. This may be useful for the elucidation of intraspecific variation in each species rather than of interspecific, intersectional or intersubsectional comparisons of chemical characters in the genus *Cirsium*.

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岩科　司，門田裕一，上野達也，大谷俊二：**日本産アザミ属植物の葉に含まれるフラボノイドとその化学分類学的意義**

今まで含有フラボノイドの報告のなかったダイ

ニチアザミ（*Cirsium babanum*），ノマアザミ（*C. chikushiense*），ホウキアザミ（*C. gratiosum*），センジョウアザミ（*C. senjoense*）およびヤマアザミ（*C. spicatum*）を含む17種の日本産アザミ属植物の葉のフラボノイドが分離された．同定できた10種類のフラボンおよびフラボノール配糖体のうち，pectolinarigenin 7-*O*-rutinoside（pectolinarin）はダイニチアザミ，ヤマアザミ，センジョウアザミなど12種の植物から主要成分として見いだされた．これはタテヤマアザミ（*C. otayae*）とビッチュウアザミ（*C. bitchuense*）を除く10種で acacetin 7-*O*-rutinoside（linarin）を伴っていた．またノマアザミ，ヤクシマアザミ（*C. yakushimense*），ツクシアザミ（*C. suffultum*）からは luteolin 7-*O*-glucoside が，イナベアザミ（*C. magofukui*）とキセルアザミ（*C. sieboldii*）からは apigenin と luteolin の 5-*O*-glucoside が主要成分として分離された．なお，ダイニチアザミでは kaempferol と quercetin の配糖体として 3-*O*-galactoside（trifolin および hyperin）および 3-*O*-arabinosylgalactoside が，ホウキアザミからは 6,8-di-*C*-glucosylapigenin（vicenin-2）が，それぞれ微量成分として同定された．

従来，分析の行われた32種のアザミ属植物から分離されたフラボノイドは，その構造の違いによって3つの型，すなわち，**A**：ポリメチル化されたフラボン（これらはすべて linarin を除いてさらに6-位も置換されている），**B**：5-位が*O*-グリコシル化されたまれなフラボン，および**C**：7-位がグリコシル化された一般的なフラボン，に大別された．A型はアグリコンのメチル化の位置と数によってさらに **A-1**: pectolinarigenin（5,7-dihydroxy-6,4′-dimethoxyflavone），**A－2**：cirsimaritin（5,4′-dihydroxy-6,7-dimethoxyflavone），および **A－3**: cirsilineol（5,4′-dihydroxy-6, 7, 3′-trimethoxyflavone）と cirsiliol（5,3′,4′-trihydroxy-6,7-dimethoxyflavone）の各型に細分された．これらのフラボノイド型は多くの場合，同一種では同一の型を示すが，カガノアザミ（*C. kagamontanum*），ビッチュウアザミ，キセルアザミ，タチアザミ（*C. inundatum*）およびチシマアザミ（*C. kamtschaticum*）では異なった型が存在し，集団間での変異がみられた．

それぞれのフラボノイド型に包含されるアザミ属の種は，多くのものについては必ずしも現在の分類体系とは一致しない．したがって，これらのフラボノイドをアザミ属の化学分類学的な指標として応用する場合，種間，節間，あるいは亜節間を区別するための指標として利用するよりもむしろ，種内変異と種分化を検討するのに適当な指標と考えられた．